MOLECULAR & CELLULAR ONCOLOGY
2019, VOL. 6, NO. 4, e1603436 (2 pages)
https://doi.org/10.1080/23723556.2019.1603436

Taylor & Francis
Taylor & Francis Group

AUTHOR'S VIEW

Insights into mitotic checkpoint by integrating CRISPR and RNAi

Gang Zhang [a,b,c]

[a]Cancer Institute, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China; [b]Qingdao Cancer Institute, Qingdao, Shandong, China; [c]Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

ABSTRACT

Our recent study of the mitotic checkpoint protein BUB1 (budding uninhibited by benzimidazoles 1) revealed several apparent *BUB1* knock-out cell lines expressing low levels of BUB1 protein sufficient to support spindle assembly checkpoint activity. This rings alarm bells on the application of CRISPR technology.

ARTICLE HISTORY
Received 19 March 2019
Revised 29 March 2019
Accepted 2 April 2019

KEYWORDS
Spindle assembly checkpoint; BUB1; ROD; CRISPR

Spindle assembly checkpoint (SAC) monitors the proper attachment of kinetochores and microtubules which guarantees the correct segregation of sister chromatids into daughter cells during mitosis. BUB1 (budding uninhibited by benzimidazoles 1) was one of the first checkpoint proteins identified in budding yeast.[1] In mammalian cells BUB1's role in the checkpoint is complicated by the fact that very little BUB1 protein is required for supporting checkpoint function.[2,3] Surprisingly, two recent studies using the gene-editing technology CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) to introduce deletions in *BUB1* upstream exons only had mild effect on checkpoint in RPE1 (retinal pigment epithelium 1) or HAP1 (a near-haploid cell line) cells.[4,5] Examined by antibody-based approaches, these cell lines appeared to lack BUB1. These studies questioned an essential role of BUB1 in the checkpoint at least in RPE1 and HAP1 cells.

In a recent study we used CRISPR to target exon 2 of *BUB1* in HeLa cells and achieved several *BUB1* knock-out cell lines. The first test on checkpoint did not show any defects consistent with the work in RPE1 and HAP1 cells. We realized however that these cells were very sensitive to *BUB1* RNAi compared to parental HeLa cells. A series of experiments suggested residual BUB1 protein expressed in these cells, which was sufficient to suport a normal checkpoint response. Proteomic analysis by mass-spectrometry identified BUB1 peptides in our *BUB1* knock-out cells covering the whole BUB1 protein except the very N-terminal end. The quantification showed around 4% BUB1 existed in the knock-out cells compared to parental cells. We then analysed the published RPE1 and HAP1 *BUB1* knock-out cells using mass-spectrometry and again identified Bub1 peptides suggesting residual BUB1 was expressed in these cell lines.[6] A parallel study from Jallepalli lab reached similar conclusions from their *BUB1* knock-out RPE1 cells and found non-sense associated alternative splicing was responsible for generating low levels of BUB1.[7] Collectively these work argues that knock-out cell lines generated by CRISPR technology should be carefully analysed and only antibody based analysis might not be enough. Since cells may still transcribe the mutated gene and the transcript may or may not be translated into functional protein, qRT-PCR (Quantitative Reverse-Transcriptase Polymerase Chain Reaction) could give misleading results. The highly sensitive mass-spectrometry is the most reliable method to validate knock-out cell lines.

Performing RNAi against *BUB1* in our *BUB1* knock-out cells reduced the mitotic timing from 600 to 110 minutes in the presence of nocodazole that depolymerises microtubules and hereby activates the checkpoint. This timing of 110 minutes is very consistent with our previous results treating parental HeLa cells with *BUB1* RNAi and expressing a dominant negative mutant of BUB1 unable to bind MAD1 (mitotic arrest deficient 1).[8] We found that depletion of the checkpoint protein ROD (rough deal), a component of the RZZ complex (ROD-ZW10-ZWILCH) that like BUB1 can bind MAD1, in cells lacking BUB1 resulted in a null checkpoint phenotype. This would support a model in which the recruitment of MAD1 by the RZZ complex can generate a reduced checkpoint signal in the complete absence of BUB1.

To further clarify the roles of RZZ and BUB1 in generating checkpoint signal, we used CRISPR to study ROD in HeLa cells. We obtained HeLa cell lines expressing less than 50% of ROD compared to parental cells after targeting *ROD* exon 2. Combined with an optimized *ROD* RNAi protocol, we were able to efficiently deplete ROD in our cell lines with reduced ROD levels. Analysing mitotic timing in these cells revealed a duration of 90 minutes in mitosis in the presence of nocodazole. This delay was dependent on BUB1 arguing that ROD and BUB1 can independently generate a reduced checkpoint signal. However we noticed that the mitotic timing in cells completely devoid of BUB1 (110 minutes) or ROD (90 minutes) is far less than 500–600 minutes which is the time parental HeLa cells spend in mitosis treated by nocodazole. This observation would favour that the integrated activities of BUB1 and ROD are required for a fully active checkpoint rather than BUB1 and RZZ work independently. As both BUB1 and the RZZ complex act to localize MAD1 to

CONTACT Gang Zhang zhanggang_sma@yahoo.com Ningxia Road 308, Qingdao, Shandong, 266071, China

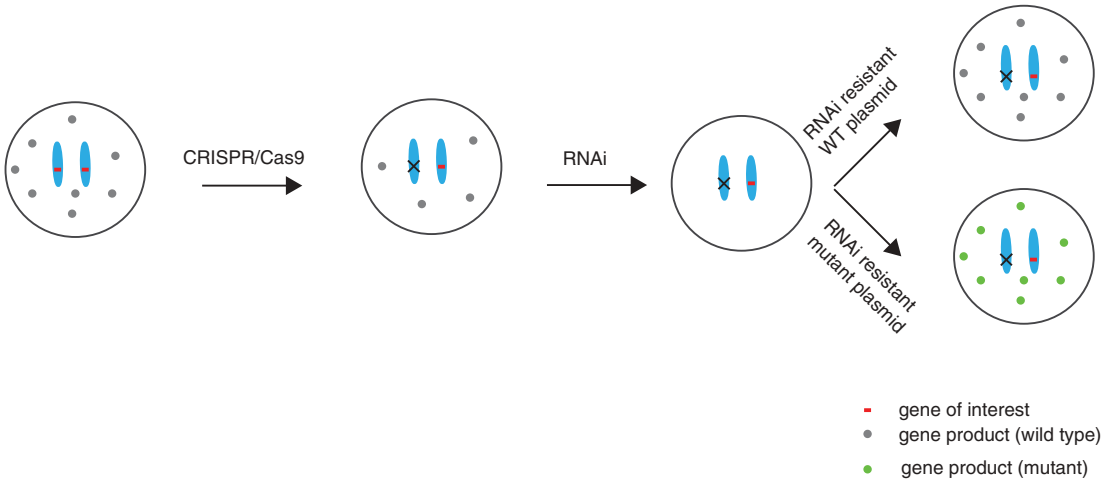

Figure 1. Rational combination of CRISPR and RNAi for studying genes of interest. Using CRISPR to generate cell lines expressing reduced amounts of protein of interest and then subsequently use RNAi to further reduce protein levels to obtain a penetrant null phenotype. This can then be integrated with expressing RNAi resistant plasmids to study the function of various domains of the protein.

unattached kinetocheres we explored in more details their roles in the SAC by artificially recruiting MAD1 to kinetochores. Strikingly this artificial recruitment of MAD1 bypassed the requirement of ROD but not BUB1 revealing a fundamental difference between the two MAD1 receptors. Further biochemical assays let us propose the role of the RZZ complex is to enrich MAD1 on kinetochores to facilitate an interaction between BUB1 and MAD1. In this model BUB1 plays an essential and possibly catalytic role in the checkpoint which needs further investigation.[6]

Collectively our work has provided important new insights into the function of BUB1 and ROD and also outlines a new strategy for analysing essential genes that are difficult to deplete using RNAi. This approach relies on generating cell lines with reduced expression of the protein of interest using CRISPR, which will result in cell lines more sensitive to RNAi depletion. The advantage of this compared to generating a full knockout cell line, which might not be possible for essential genes, is that the cell lines might not adapt during the selection process which has been observed.[9] These CRISPR cell lines can then be integrated with RNAi depletion protocols and phenotypes rescued by expressing RNAi resistant plasmids allowing researchers to study the function of individual domains or modifications of the protein (Figure 1).

Acknowledgments

ZG wants to thank all the co-authors for the contribution to the original work and Jakob Nilsson for the comments of this manuscript. ZG is supported by Taishan Scholar Project of Shandong Province (tsqn201812054), China.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the Taishan Scholar Project of Shandong Province [tsqn201812054].

ORCID

Gang Zhang http://orcid.org/0000-0001-7697-7203

References

1. Hoyt MA, Totis L, Roberts BT. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. Cell. 1991;66:507–517.
2. Johnson VL, Scott MI, Holt SV, Hussein D, Taylor SS. Bub1 is required for kinetochore localization of BubR1, Cenp-E, Cenp-F and Mad2, and chromosome congression. J Cell Sci. 2004;117:1577–1589. doi:10.1242/jcs.01006.
3. Meraldi P, Sorger PK. A dual role for Bub1 in the spindle checkpoint and chromosome congression. EMBO J. 2005;24:1621–1633. doi:10.1038/sj.emboj.7600641.
4. Currie CE, Mora-Santos M, Smith CA, McAinsh AD, Millar JBA. Bub1 is not essential for the checkpoint response to unattached kinetochores in diploid human cells. Curr Biol. 2018;28:R929–R930. doi:10.1016/j.cub.2018.07.040.
5. Raaijmakers JA, van Heesbeen RGHP, Blomen VA, Janssen LME, van Diemen F, Brummelkamp TR, Medema RH. Bub1 is essential for the viability of human cells in which the spindle assembly checkpoint is compromised. Cell Rep. 2018;22:1424–1438. doi:10.1016/j.celrep.2018.01.034.
6. Zhang G, Kruse T, Guasch Boldú C, Garvanska DH, Coscia F, Mann M, Barisic M, Nillson J. Efficient mitotic checkpoint signaling depends on integrated activities of Bub1 and the RZZ complex. EMBO J. 2019;pii:e100977. doi:10.15252/embj.2018100977.
7. Rodriguez-Rodriguez JA, Lewis C, McKinley KL, Sikirzhytski V, Corona J, Maciejowski J, Khodjakov A, Cheeseman IM, Jallepalli PV. Distinct roles of RZZ and Bub1-KNL1 in mitotic checkpoint signaling and kinetochore expansion. Curr Biol. 2018;28:3422–3429,e5. doi:10.1016/j.cub.2018.10.006.
8. Zhang G, Kruse T, López-Méndez B, Sylvestersen KB, Garvanska DH, Schopper S, Nielsen ML, Nilsson J. Bub1 positions Mad1 close to KNL1 MELT repeats to promote checkpoint signalling. Nat Commun. 2017;8:15822. doi:10.1038/ncomms15822.
9. Wild T, Larsen MS, Narita T, Schou J, Nilsson J, Choudhary C. The spindle assembly checkpoint is not essential for viability of human cells with genetically lowered APC/C activity. Cell Rep. 2016;14:1829–1840. doi:10.1016/j.celrep.2016.01.060.